

CLAIMS

This complete listing of the claims, with updated status indicators, is provided below:

Claims 1. - 525. (Canceled)

526. (New) A reporter signal peptide from about 10 to about 35 amino acids comprising a single amino acid sequence Asp-Pro, wherein the reporter signal peptide can be fragmented across the Asp-Pro peptide bond by collision-induced dissociation in an ion trap mass spectrometer.

527. (New) The reporter signal peptide of claim 526, further comprising a coupling agent for covalent coupling to a protein or a peptide.

528. (New) The reporter signal peptide of claim 527, wherein the coupling agent comprises a chemically reactive group.

529. (New) The reporter signal peptide of claim 528, wherein the coupling agent further comprises a linker linking the chemically reactive group to the reporter signal peptide.

530. (New) The reporter signal peptide of claim 528, wherein the chemically reactive group can covalently couple with a free sulfhydryl group of a cysteine residue.

531. (New) The reporter signal peptide of claim 529, wherein the chemically reactive group is selected from the group consisting of thiols, epoxides, or nitriles.

532. (New) The reporter signal peptide of claim 528, wherein the chemically reactive group can react with a free amino-terminal primary amino group of a protein or a peptide.

533. (New) The reporter signal peptide of claim 532, wherein the chemically reactive group is selected from the group consisting of an NHS ester and an isothiocyanate.

534. (New) The reporter signal peptide of claim 533, wherein the chemically reactive group is an NHS ester.

535. (New) The reporter signal peptide of claim 528, wherein the coupling agent further comprises a linker.

536. (New) The reporter signal peptide of claim 526 comprising the sequence (Aa)_n-Asp-Pro-(Aa)_m, wherein Aa is an amino acid residue and n and m are each independently an integer of 2 or more and the sum of n + m is about 8 to about 33 amino acids.

537. (New) The reporter signal peptide of claim 536, wherein the reporter signal peptide sequence is selected from the group consisting of SEQ ID NO:24, SEQ ID NO: 25 and SEQ ID NO: 26.

538. (New) The reporter signal peptide of claim 526 comprising the sequence (Aa)_n-Asp-Pro-(Aa)_m, wherein n and m is each independently an integer from 2 to 5 and Aa is an amino acid residue.

539. (New) The reporter signal peptide of claim 538, wherein each amino acid residue Aa is independently selected from the group consisting of glycine, alanine, valine, leucine, and isoleucine.

540. (New) The reporter signal peptide of claim 539, wherein each amino acid residue Aa is the same amino acid residue.

541. (New) The reporter signal peptide of claim 540, wherein the amino acid residue Aa is glycine.

542. (New) The reporter signal peptide of claim 539, wherein m is equal to n.

543. (New) The reporter signal peptide of claim 526, wherein at least one amino acid is modified by isotopic enrichment, methylation, phosphorylation, sulphation, and use of selenomethionine for methionine.

544. (New) The reporter signal peptide of claim 543, wherein the at least one modified amino acid is modified by isotopic enrichment.

545. (New) The reporter signal peptide of claim 544, wherein isotopic enrichment comprises a ¹³C atom, a ¹⁵N atom, a deuterium atom, or any combination thereof.

546. (New) A set of reporter signal peptides comprising two or more reporter signal peptides of claim 526, wherein each of the reporter signal peptides has the same molecular mass.

547. (New) The set of reporter signal peptides of claim 546, wherein each of the reporter signal peptides has the same mass-to-charge ratio following ionization in a mass spectrometer.

548. (New) The set of reporter signal peptides of claim 547, wherein the mass-to-charge ratio of each fragmented reporter signal peptide in the set can be distinguished from the mass-to-charge ratio of the other fragmented reporter signal peptides in the set.

549. (New) The set of reporter signal peptides of claim 548, wherein the reporter signal peptides further comprise a coupling agent having a chemically reactive group for covalent coupling to a target protein or peptide.

550. (New) The set of reporter signal peptides of claim 549, wherein the chemically reactive group covalently couples a free sulfhydryl group of the target protein or peptide.

551. (New) The set of reporter signal peptides of claim 550, wherein the chemically reactive group is selected from the group consisting of: a thiol, an epoxide, and a nitrile.

552. (New) The set of reporter signal peptides of claim 549, wherein the chemically reactive group covalently couples an amino-terminal primary amine group of the target protein or peptide.

553. (New) The set of reporter signal peptides of claim 552, wherein the chemically reactive group is selected from the group consisting of: an NHS ester, and an isothiocyanate.

554. (New) The set of reporter signal peptides of claim 546, wherein the set of reporter signals comprises two or more of CG*G*G*G*DPGGGGR (SEQ ID NO: 1), CG*G*G*GDPGGGG*R (SEQ ID NO: 1), CG*G*GGDPGGG*G*R (SEQ ID NO: 1), CG*GGGDPGG*G*G*R (SEQ ID NO: 1), and CGGGGDPG*G*G*G*R (SEQ ID NO: 1), wherein each G* is a glycine with at least one heavy isotope.

555. (New) A method comprising:

labeling a protein or a peptide in a sample with a reporter signal peptide according to claim 526;

separating the labeled protein or peptide or fragments thereof from molecules having a different mass-to-charge ratio in a mass spectrometer;

fragmenting the reporter signal peptide by collision induced dissociation in an ion trap mass spectrometer; and

detecting fragmented reporter signal peptide.

556. (New) The method of claim 555, further comprising quantifying the amount of the fragmented reporter signal peptide.

557. (New) The method of claim 556, further comprising comparing the amount of the fragmented reporter signal peptide to a known or an expected value.

558. (New) The method of claim 555, further comprising denaturing the protein or peptide prior to labeling it with the reporter signal peptide.

559. (New) The method of claim 555, further comprising producing the sample by a separation procedure.

560. (New) The method of claim 559, wherein the separation procedure is selected from the group consisting of liquid chromatography, gel electrophoresis, two-dimensional chromatography, two-dimensional gel electrophoresis, isoelectric focusing, thin layer chromatography, centrifugation, filtration, ion chromatography, immunoaffinity chromatography, membrane separation, and a combination thereof.

561. (New) The method of claim 555, further comprising fragmenting the labeled protein or peptide before separating the labeled protein or peptide or fragments thereof in a mass spectrometer.

562. (New) The method of claim 561, wherein the labeled protein or peptide is fragmented by digestion with a protease.

563. (New) The method of claim 562, wherein the protease is trypsin.

564. (New) The method of claim 555, wherein the reporter signal peptide comprises one or more of CG*G*G*G*DPGGGGR (SEQ ID NO: 1), CG*G*G*GDPGGGG*R (SEQ ID NO: 1), CG*G*GGDPGGG*G*R (SEQ ID NO: 1), CG*GGGDPGG*G*G*R (SEQ ID NO: 1), CGGGGDPG*G*G*G*R (SEQ ID NO: 1), wherein each G* is a glycine with at least one heavy isotope.

565. (New) A method comprising:

labeling a set of proteins or peptides in a sample with a set of reporter signal peptides according to claim 546;

separating the set of labeled proteins or peptides or fragments thereof from molecules having a different mass-to-charge ratio in a mass spectrometer;

fragmenting the reporter signal peptides by collision induced dissociation in an ion trap mass spectrometer; and

detecting fragmented reporter signals; and

distinguishing the fragmented reporter signal peptides from each other.

566. (New) The method of claim 565, further comprising quantifying the amount of a first fragmented reporter signal peptide.

567. (New) The method of claim 566, further comprising quantifying the amount of a second fragmented reporter signal peptide.

568. (New) The method of claim 567, further comprising comparing the amounts of the first and the second fragmented reporter signal peptides.

569. (New) The method of claim 565, wherein the sample is a complex sample comprising multiple proteins.

570. (New) The method of claim 565, further comprising producing the sample by a separation procedure.

571. (New) The method of claim 570, wherein the separation procedure is selected from the group consisting of liquid chromatography, gel electrophoresis, two-dimensional chromatography, two-dimensional gel electrophoresis, isoelectric focusing, thin layer chromatography, centrifugation, filtration, ion chromatography, immunoaffinity chromatography, membrane separation, and a combination thereof.

572. (New) The method of claim 565, further comprising denaturing the set of proteins or peptides prior to labeling them with the set of reporter signals.

573. (New) The method of claim 565, further comprising fragmenting the labeled proteins or peptides before separating the set of labeled proteins or peptides or fragments thereof in a mass spectrometer.

574. (New) The method of claim 565, wherein the labeled proteins or peptides are fragmented by digestion with a protease.

575. (New) The method of claim 565, wherein the protease is trypsin. .

576. (New) The method of claim 565, wherein the set of reporter signal peptides comprises one of more of CG*G*G*G*DPGGGGR (SEQ ID NO: 1), CG*G*G*GDPGGGG*R (SEQ ID NO: 1), CG*G*GGDPGGG*G*R (SEQ ID NO: 1), CG*GGGDPGG*G*G*R (SEQ ID NO: 1), CGGGGDPG*G*G*G*R (SEQ ID NO: 1), wherein each G* is a glycine with at least one heavy isotope.

577. (New) A kit comprising:

the set of reporter signal peptides according to claim 546; and

a set of instructions for use.

578. (New) The kit of claim 577, further comprising at least one target peptide labeled with a reporter signal peptide of claim 526.

579. (New) The kit of claim 578, wherein the protein or peptide comprises a cysteine amino acid residue.

580. (New) The kit of claim 577, wherein the set of reporter signals comprises one of more of CG*G*G*G*DPGGGGR (SEQ ID NO: 1), CG*G*G*GDPGGGG*R (SEQ ID NO: 1), CG*G*GGDPGGG*G*R (SEQ ID NO: 1), CG*GGGDPGG*G*G*R (SEQ ID NO: 1), CGGGGDPG*G*G*G*R (SEQ ID NO: 1), wherein each G* is a glycine with at least one heavy isotope.

581. (New) A protein or peptide labeled with a reporter signal peptide of claim 526.

582. (New) The protein or peptide of claim 581, wherein the protein or peptide comprises a cysteine amino acid residue.

583. (New) A set of two or more labeled proteins or peptides according to claim 581.

584. (New) A set of labeled peptides or proteins labeled with the set of reporter signal peptides of claims 546.

585. (New) A set of labeled peptides or protein labeled with a set of reporter signals, wherein the set of reporter signals comprises one of more of CG*G*G*G*DPGGGGR (SEQ ID NO: 1), CG*G*G*GDPGGGG*R (SEQ ID NO: 1), CG*G*GGDPGGG*G*R (SEQ ID NO: 1), CG*GGGDPGG*G*G*R (SEQ ID NO: 1), CGGGGDPG*G*G*G*R (SEQ ID NO: 1), wherein each G* is a glycine with at least one heavy isotope.